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How do soil emissions of N_2O, CH_4 and CO_2 from perennial bioenergy crops differ from arable annual crops?

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Abstract

It is important to demonstrate that replacing fossil fuel with bioenergy crops can reduce the national greenhouse gas (GHG) footprint. We compared field emissions of nitrous oxide (N_2O), methane (CH_4) and soil respiration rates from the C_4 grass *Miscanthus* × *giganteus* and willow (*salix)* with emissions from annual arable crops grown for food production. The study was carried out in NE England on adjacent fields of willow, *Miscanthus*, wheat (*Triticum aetivum*) and oilseed rape (*Brassica napus*). N_2O, CH_4 fluxes and soil respiration rates were measured monthly using static chambers from June 2008 to November 2010. Net ecosystem exchange (NEE) of carbon dioxide (CO_2) was measured by eddy covariance on *Miscanthus* from May 2008 and on willow from October 2009 until November 2010. The N_2O fluxes were significantly smaller from the bioenergy crops than that of the annual crops. Average fluxes were 8 and 32 μg m^{-2} h^{-1} N_2O-N from wheat and oilseed rape, and 4 and 0.2 μg m^{-2} h^{-1} N_2O-N from *Miscanthus* and willow, respectively. Soil CH_4 fluxes were negligible for all crops and soil respiration rates were similar for all crops. NEE of CO_2 was larger for *Miscanthus* (−770 g C m^{-2} h^{-1}) than willow (−602 g C m^{-2} h^{-1}) in the growing season of 2010. N_2O emissions from *Miscanthus* and willow were lower than for the wheat and oilseed rape which is most likely a result of regular fertilizer application and tillage in the annual arable cropping systems. Application of ^{15}N-labelled fertilizer to *Miscanthus* and oil seed rape resulted in a fertilizer-induced increase in N_2O emission in both crops. Denitrification rates (N_2O + N_2) were similar for soil under *Miscanthus* and oilseed rape. Thus, perennial bioenergy crops only emit less GHGs than annual crops when they receive no or very low rates of N fertilizer.

Keywords: bioenergy, methane, Miscanthus, nitrous oxide, short rotation coppice, soil respiration, willow

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Introduction

To provide low-carbon (C) energy in the UK, Europe and worldwide, biomass is increasingly used as a renewable resource (Thornley *et al*., 2009). For example, the EU has set a target of producing 20% (UK 15%) of the energy from renewable sources, of which biomass should contribute a significant part, by 2020. The UK is a signatory to this agreement (Great Britain, 2008b) and the UK government has recently introduced plans, as the Climate Change Act 2008, to reduce greenhouse gas (GHG) emissions by 80% over 1990 levels by 2050 with specific progress to be made by 2020 to tackle climate change and energy security (Great Britain, 2008a). To meet these targets the UK bioenergy industry will have to expand significantly to be able to supply enough energy feedstocks. For example, the contribution of renewable energy to power generation will have to rise from about 3.1% to about 20% in 2020 as mentioned in the Energy White Paper (DEFRA, 2007). A more recent report suggests the expansion of perennial energy crops to around 350 000 hectares by 2020. This would yield a total of land available for energy production including biofuels with biomass crops, of around a million hectares which equals 17% of the UK arable land (Atkinson, 2009). Current UK policy commitment is to increase the land given over to bioenergy crops, justified mainly by the benefit in atmospheric carbon dioxide (CO_2) emissions compared with combustion of fossil fuels. However, crops and their agricultural management regimes have much wider impact on total GHG budgets to the atmosphere than just CO_2. For example, the gases nitrous oxide (N_2O) and methane (CH_4), whose fluxes

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are sensitive to soil conditions, have global warming potentials (GWPs) tens of times that of CO_2 (IPCC, 2007).

In temperate climates, such as the UK, the two bioenergy crops that are most promising alternatives to fossil fuels are *Miscanthus* × *giganteus* (a perennial rhizomatous C_4 grass) and short rotation coppice (SRC) willow (C_3). They are currently grown commercially on a total of ~14 000 ha in the UK, and are mainly used either for co-firing in electricity generation or for local combined heat and power. The development of second generation biofuel production, based on lignocellulose, is expected to be a major driving force for the expansion of these bioenergy crops.

Most of the recent published studies are the Life Cycle Analyses (LCA) for bioenergy crops (Hillier *et al*., 2009; Whitaker *et al*., 2010; Brandão *et al*., 2011) or modelling studies of C fluxes (Dondini *et al*., 2009). Actual longer-term field GHG measurement studies are sparse and there have been only few reports on GHG emissions from these bioenergy crops, but information on their physiology, nitrogen (N) and water requirements and management lead us to hypothesize that emissions will differ compared with annual cropping. So, it is important to demonstrate that replacing fossil fuel with these 'new' bioenergy crops can reduce the national GHG footprint. Data are also needed to improve the reliability of LCAs of bioenergy chains, which are mostly based on standard emission factors for N_2O or default values (IPCC Tier 1) for soil organic C dynamics. The data sets from which these default values originate do not involve lignocellulosic crops. We therefore studied the emissions of soil N_2O, CH_4 and CO_2 during growth of *Miscanthus* and SRC willow for 2½ years and compared these with emissions from annual arable crops grown for food production (from now on referred to as 'annual crops').

SRC willow and *Miscanthus* are physiologically different from current arable crops (Karp & Shield, 2008): they are perennial with economic lifespans of up to 20 years, deep rooted (~2 m) with a generally greater root biomass (Neukirchen *et al*., 1999), conferring high water-use efficiency (Liebig *et al*., 2005), and have tall canopies. They are adapted to more acidic conditions than annual crops and so do not require liming. Root-derived C flow in *Miscanthus* is thought to be moderated by the rhizome, which offers the potential for belowground storage of both C and N (Heaton *et al*., 2004). In addition to this, litter decomposition is likely to be slower than that in annual arable crops due to the absence of disturbance through cultivation. In this sense, planting of perennial bioenergy crops, whether perennial grasses or SRC willow, can be considered analogous to conversion of arable land to pasture or forestry. Management of perennial bioenergy crops differs to that of annual crops. SRC willow is harvested on a 3 year cycle, at which time the canopy is typically 8 m high. *Miscanthus* is harvested annually, generally in spring, and is typically 3–4 m in height. *Miscanthus* is unusual because it is able to use the C_4 photosynthetic pathway at a significantly lower temperature than most C_4 plants (Naidu *et al*., 2003) and is therefore ideally suited to temperate climates such as the UK. The use of nutrients and water is very efficient and perennial energy yields for *Miscanthus* reported for Northern Europe match or exceed those reported for SRC willow. Potentially N application has only a limited effect on the yield of *Miscanthus* (Danalatos *et al*., 2007) or no effect (Strullu *et al*., 2011). Willow, on the other hand, does respond to inputs of N, with an annual biomass production of 15–20 Mg DM ha^{-1} removing 75 kg N ha^{-1} yr^{-1} (Naidu *et al*., 2003). As a result, unlike high N demanding bioenergy crops, such as oilseed rape and maize, *Miscanthus* and SRC willow do not require high rates of N fertilization and so direct fertilizer-induced emissions of N_2O are hypothesized to be lower than those for N demanding crops (Crutzen *et al*., 2008).

Miscanthus is believed to have a high potential biomass production with a low nitrogen (N) requirement (Lewandowski *et al*., 2000). In the context of GHG savings, the N requirement is a very sensitive issue as it is known that manufacture of N-fertilizers is very energy-intensive and are therefore strongly affecting the total energy balance of crops (Boehmel *et al*., 2008). In addition, losses of gaseous N_2O after fertilization have a wide impact. According to existing literature, there is no consensus yet about the N-fertilizer requirement of *Miscanthus* although biomass production has been described as being dependent on air temperature, precipitation and soil water availability (Clifton-Brown *et al*., 2004; Richter *et al*., 2008). Although some authors report that N-fertilization is needed to achieve maximum biomass production (Ercoli *et al*., 1999; Cosentino *et al*., 2007; Boehmel *et al*., 2008), others propose that biomass production of *Miscanthus* is not related to N-fertilization at all (Himken *et al*., 1997; Clifton-Brown *et al*., 2007; Danalatos *et al*., 2007). The consensus appears to be that the N requirement of *Miscanthus* is low compared with that of other crops due to efficient N-recycling within the crop (Lewandowski & Schmidt, 2006). In spring, part of the rhizome nitrogen stocks are remobilized from belowground to aboveground organs, and in autumn nitrogen accumulated in the aboveground is translocated from aboveground to belowground organs as the plant senesces (Himken *et al*., 1997; Christian *et al*., 2006). The factors that affect these remobilizations are not yet known and rates

reported in the literature vary substantially. For example, plot experiments in Northern France showed spring remobilization in the order of 52–141 kg N ha^{-1} and autumn remobilization between 39 and 145 kg N ha^{-1} (Strullu *et al*., 2011). It has also been postulated that N_2 fixation occurs in *Miscanthus* in association with the N_2-fixing *Azospirillum* (Eckert *et al*., 2001).

Applying standard IPCC Tier I methodology to calculate GHG fluxes from *Miscanthus* and SRC willow suggests that due to their low demand for N, N_2O emissions are expected to be less than for many agricultural crops (IPCC, 2007). However, in Denmark, 1.09 kg N_2O-N ha^{-1} yr^{-1} from sandy loam soil under a *Miscanthus* crop were measured, which was about twice that emitted from under winter rye (Jørgensen *et al*., 1997); both crops were fertilized, the *Miscanthus* plot was harvested and fertilized with 75 kg N ha^{-1} annually for 6 years whereas the rye plot was fertilized with 120 kg N ha^{-1}. This highlights the need for measurements from these systems. As the *Miscanthus* plots in Denmark were about the same age as the ones investigated in this study, the main difference between the results from Denmark and the ones reported here appears to be that the *Miscanthus* plots in Denmark were fertilized. To correctly assess the impact of perennial bioenergy crops on climate change, and to propose appropriate mitigation strategies, it is essential to study the three main GHGs during crop growth simultaneously and to understand the underlying microbial processes involved in the production and net soil emissions of CO_2, N_2O and CH_4.

Hypothesis

We hypothesize that GHG emissions from soil under bioenergy cropping will significantly differ from that under annual cropping due to the lower N requirement of bioenergy crops.

Materials and methods

This study was conducted at a commercial farm in Lincolnshire, NE England (53°18′55″N; 0°34′40″W) on adjacent fields of willow (*Salix spec*.), *Miscanthus* (*Miscanthus* × *giganteus*), wheat (*Triticum aestivum*) and oilseed rape (*Brassica napus*). The soil association is Beccles 1, fine loam over clay and the bedrock is Charnmouth mudstone formation. The soil pH was 7.13 ± 0.02 (standard deviation (SD) of 3 replicates) in the *Miscanthus* and 5.84 ± 0.26 in the willow, 6.32 ± 0.68 in annual crop A and 6.45 ± 0.09 in annual crop B (Table 1). Total C was 1.22% in *Miscanthus* and 2.18% in willow and total N 0.3% and 0.37% respectively. Bulk density was 1.43 ± 0.06 g cm^{-3} in *Miscanthus*, 1.35 ± 0.24 g cm^{-3} in willow and 1.22 ± 0.06 g cm^{-3} and 1.55 ± 0.061 g cm^{-3} in the two annual arable crops (a wheat oil seed rape rotation) respectively (Table 1). The long-term mean annual minimum temperature (from 1971 to 2000) was 5.9°C and the long-term mean annual maximum temperature was 13.1°C. Mean annual rainfall was 605 mm [Scampton (nearby site) over 25 years from 1963 to 2004]. The average total N deposition (2006–2008) was 12.16 kg ha^{-1} yr^{-1} (R. Smith, personal communication). The four fields lie next to each other, the terrain and climate are the same. Prior conversion to perennial crops, the *Miscanthus* and willow fields were farmed in exactly the same way as the annual crop fields which generally is a crop rotation of 3 years of wheat and 1 year of oilseed rape. Directly prior to conversion, both bioenergy crop fields had 3 years of wheat cultivation.

Short rotation coppice Salix (SRC willow) was established in 2002 and is managed on a 3 year cropping cycle with harvest taking place in the autumn. It was first harvested in October 2007. The high-density willow plantation was established with around 15 000 stools ha^{-1} of five different varieties planted at random to avoid spreading of diseases. The *Miscanthus* was established in 2005, with a planting density of 10 000 rhizomes ha^{-1}. It is harvested in the spring which occurred for the first time 2007. The energy crops grown at this field site are used for co-firing in a power station. The two annual crop fields sampled during this study were a winter wheat – oilseed rape crop rotation. Further, they will be referred to as annual crop A (ACA) which had a crop rotation of wheat – wheat – oilseed rape during the measurement

Table 1 Summary of soil parameters of the different crops (SD = standard deviation)

	pH	SD[a]	Bulk density [g cm^{-3}]	SD[b]	Soil NO_3 [μg g^{-1}]	SD[c]	Soil NH_4 [μg g^{-1}]	SD[c]	Soil moisture [vol %]	SD[d]
Miscanthus	7.13	0.02	1.43	0.06	0.08	0.04	3.99	2.70	30.93	6.22
Willow	5.84	0.26	1.35	0.24	0.25	0.19	2.54	1.55	29.75	8.62
ACA	6.32	0.68	1.55	0.06	0.66	0.06	1.20	0.45	30.35	11.31
ACB	6.45	0.09	1.22	0.06	1.25	0.19	1.40	0.28	27.14	9.08

[a]SD = three replicates, soil taken May 2011.
[b]SD = five replicates, soil taken February 2010.
[c]SD = three replicates, soil taken February 2010.
[d]SD = 99, average over measurement period from June 2008 to November 2010.

period and annual crop B (ACB) which had a crop rotation of oilseed rape – wheat – oilseed rape during the 3 years. The bioenergy crops were planted on former wheat (3 years) fields and did not receive any N fertilizer in the duration of the project or during the establishment phase. The only disturbance was caused by the annual harvests for *Miscanthus* and three yearly harvest for willow. In contrast, the annual crops received three applications of N-fertilizer every spring (35/70/35 kg N ha^{-1} equalling 140 kg N ha^{-1} yr^{-1}), they were harvested in August, and the new crop was directly drilled into the stubble field in early September. Peak emission events associated with N fertilization and ploughing were avoided in this monthly comparison of fluxes from the bioenergy and annual crop fields.

The N_2O and CH_4 flux measurements were made using static chambers, soil respiration rates by dynamic chambers and the net ecosystem exchange (NEE) of CO_2 was measured continuously by eddy covariance, the latter only on the biomass crops. In each crop, [*Miscanthus*, SRC willow and annual crops (ACA = wheat-wheat-oilseed rape and ACB = oilseed rape-wheat-oilseed rape)] five chambers were established in June 2008 and subsequently sampled monthly until November 2010. Flux towers for eddy covariance measurements were set up in the *Miscanthus* in May 2008 and in the SRC willow in October 2009. Unfortunately, due to lack of this very expensive equipment, such measurements could not be made in the annual crop fields. Flux measurements of CH_4 and N_2O were carried out using a static chamber method (Clayton *et al*., 1994). The round chambers (d = 40 cm) consisted of opaque polypropylene bases inserted into the ground to a depth of approximately 5 cm and the bases remained *in situ* and aluminium lids were only put on during the enclosure of 60 min. A three way tap allowed easy and quick gas sample removal. The enclosure volume was approximately 20 litres (h = 16 cm). Gas samples were taken into 500 ml Tedlar bags (SKC Ltd., Dorset, UK) using a 100 ml syringe. Subsequently, samples were analysed at CEH Edinburgh on an HP5890 Series II gas chromatograph [Hewlett Packard (Agilent Technologies) UK Ltd., Stockport, UK] with electron capture detector (ECD) and flame ionization detector (FID) for N_2O and CH_4 analysis respectively. GC accuracy was 30 ppb for N_2O and 70 ppb for CH_4. Fluxes were calculated as the observed rate of concentration change (using three time steps) times the enclosure volume to ground surface ratio. The monthly averages were used to estimate the annual gas balances from chamber flux measurements.

A standard method for CO_2 flux measurements by eddy covariance was used (Lloyd, 2006). CO_2 fluxes were measured using a LiCor 7500 (Li-Cor, Inc., Lincoln, Nebraska, USA). Extendable hydraulic masts were used to maintain the sensor head at a height of 2 m above the top of the fast growing crops. Fluxes were calculated, and gap-filled (Papale *et al*., 2006). In addition, continuous measurements of the air temperature were made (platinum resistance thermometer, Didcot Instruments Ltd), from an automatic weather station situated adjacent to the crops, and soil temperature (Thermistor Model PT107, Campbell Scientific Ltd.) at a depth of 10 cm. In both cases, the values were data logged as 30 minute averages.

Soil respiration was measured using a small (0.001171 m^3) dynamic chamber covering an area of 0.0078 m^2 of soil for 120 s. Sample analysis was immediate using an EGM-4 infrared gas analyser (IRGA) (PP Systems; Hitchin, Hertfordshire, England). Usually soil respiration measurements were carried out over soil adjacent to the static chambers during their enclosure time. Volumetric soil moisture content was measured at 10 cm depth with a Theta probe HH 2 moisture meter (Delta T-Devices, Cambridge, England) at three points around the static chambers at each time of enclosure, which was calibrated against gravimetric measurements.

A stable isotope experiment was carried out in September 2010, to investigate total denitrification loss of gaseous N in the C_4 *Miscanthus* plantation and compare these with denitrification losses from the recently harvested oilseed rape field (C_3). $^{14}NH_4{}^{15}NO_3$ fertilizer was applied to three replicate plots in *Miscanthus* and oilseed rape (ACB; which was just sown into nontilled soil). An equivalent of 50 kg N ha^{-1} of $^{14}NH_4{}^{15}NO_3$ at 20 atom% ^{15}N in solution was applied to 1 m^2 plots and then the chambers were inserted into the middle of the plots. Gas samples were taken before and almost daily for 8 days after fertilization, and were analysed for ^{15}N-N_2O and ^{15}N-N_2 on an Isotope ratio mass spectrometer (SerCon Ltd.) at the University of Aberdeen following cryofocusing in an ANCA TGII trace gas preparation system. Soil samples were also analysed for ^{15}N-enrichment of the NH_4 and NO_3 pools on the isotope ratio mass spectrometer after extraction with 2 M KCl and microdiffusion of the ^{15}N pools (Brooks *et al*., 1989).

For statistical analysis the R statistical software package (R.2.10.1) was used, applying linear mixed effects models to the N_2O flux data (Pinheiro *et al*. 2009). To achieve normality, a constant of 20 was added to each data point and the dataset was then log transformed. Fixed effect was crop type and random effect was date to account, for multiple measurements over time. Each 'crop type' consisted of about 230 individual measurements. The significance of the model terms were assessed using a Likelihood Ratio test.

Results

Variations in N_2O and CH_4 fluxes and soil respiration rates throughout the study period are shown in Fig. 1. N_2O emissions were about a factor 5 larger from the annual crops than the perennial bioenergy crops. (Fig. 1a, note scale is an order of magnitude smaller on Fig. 1(a2) to clarify temporal variation of the bioenergy crops). For Miscanthus, we observed notably larger N_2O emissions in May 2009, however, these were smaller than from the annual crops and occurred in three of the five flux chambers. The reason for these higher fluxes cannot be explained. Mean N_2O fluxes over the 2½ years measuring period were 3.7 μg N_2O-N m^{-2} h^{-1} from the *Miscanthus* field, 0.2 μg N_2O-N m^{-2} h^{-1} from willow, 8.3 μg N_2O-N m^{-2} h^{-1} from ACA (wheat-wheat-oilseed rape) and 32.3 μg N_2O-N m^{-2} h^{-1} from ACB (oilseed rape-wheat-oilseed rape) respectively (Fig. 1;

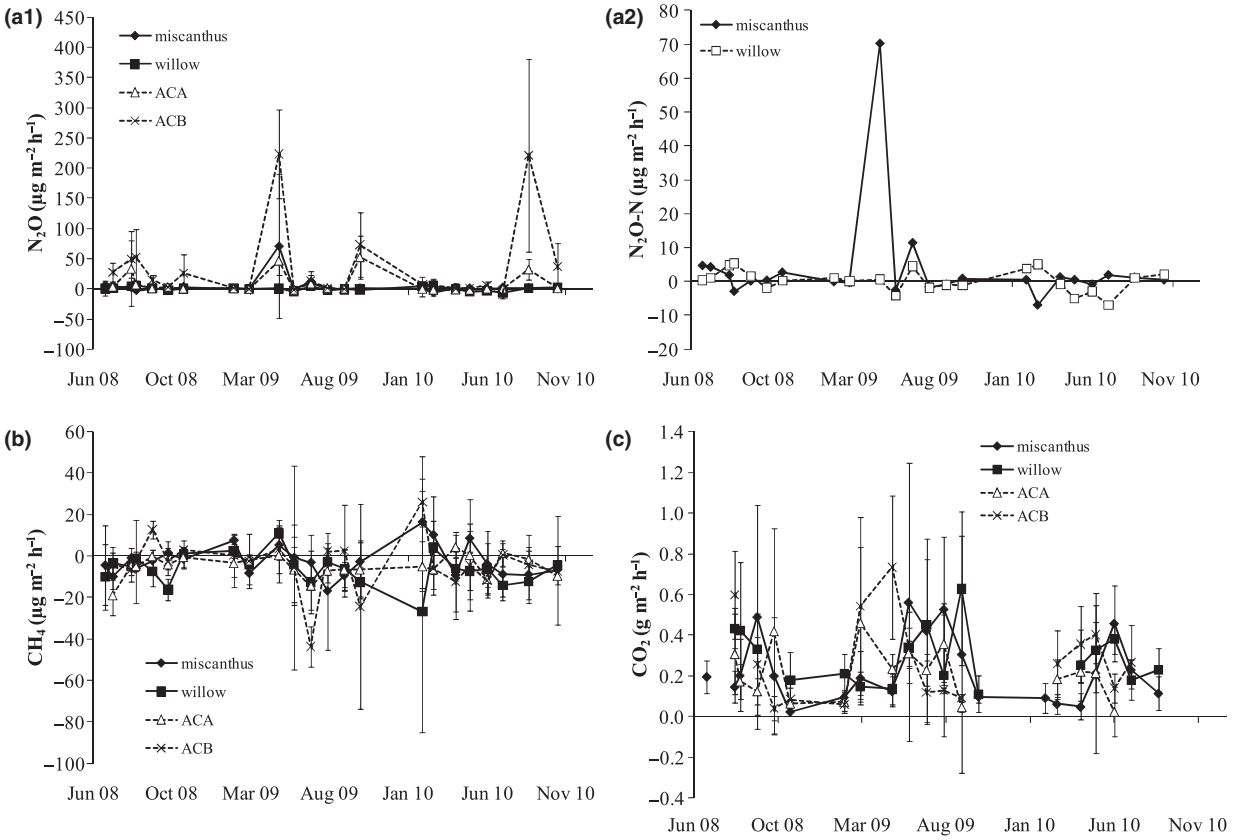

Fig. 1 Seasonal (a), N_2O, (b) CH_4, and soil respiration (c) fluxes from *Miscanthus*, willow and the annual crops. A2 shows N_2O emissions from the bioenergy plots at a magnified scale. Error bars are standard deviations from replicate chambers per plot (n = 5).

Table 2). Minimum, maximum and median fluxes are summarized in Table 2.

Interpretation of linear mixed effects models with willow and *Miscanthus* as group A (bioenergy crops) and the annual crops ACA and ACB as group B (annual crops), showed that N_2O emissions were significantly different from bioenergy crops compared with the annual crops (Likelihood ratio test, $L = 66.75$, $P < 0.0001$), but differences between the willow and *Miscanthus* crop or the annual crops ACA and ACB were not significant.

There were no clear trends between different crops in terms of CH_4 emissions, but generally CH_4 uptake took place which was indicative of oxidation (negative fluxes) (Fig 1b). Mean CH_4 fluxes were −2.5 and −6.5 µg m^{-2} h^{-1} from *Miscanthus* and willow respectively and −5 and −4 µg m^{-2} h^{-1} from the two annual crops, minimum, maximum and median fluxes as shown in Table 2.

Soil respiration rates showed a clear seasonal trend with higher emissions in summer than that in winter for all crops (Fig. 1c), but there were no significant differences between soil respiration rates in soil under bioenergy (0.23 and 0.3 g m^{-2} h^{-1} from *Miscanthus* and willow) or annual crops (0.21 and 0.29 g m^{-2} h^{-1}) (Table 2). However, spring fluxes appear to be larger in the annual crops which might be due to fertilizer application.

Table 2 Summary of GHG fluxes in the bioenergy and annual arable crops

	Mean	Median	Min	Max	SD	n
N_2O (µg N_2O-N m^{-2} h^{-1})						
Miscanthus	3.7	−0.2	−11	275	27.1	114
Willow	0.2	0.8	−16	33	6.0	144
ACA	8.3	0.7	−9	144	22.2	109
ACB	32.3	4.8	−15	455	70.4	110
CH_4 (µg m^{-2} h^{-1})						
Miscanthus	−2.5	−2.9	−59	69	14.8	114
Willow	−6.5	−5.4	−128	29	15.6	144
ACA	−5	−2.6	−52	26	11.2	109
ACB	−4	−2.7	−101	114	20.7	110
Soil respiration CO_2 (g m^{-2} h^{-1})						
Miscanthus	0.23	0.14	−0.06	1.76	0.30	104
Willow	0.3	0.25	−0.02	1.24	0.22	83
ACA	0.21	0.17	−0.17	1.31	0.23	80
ACB	0.29	0.21	0	1.35	0.27	75

To estimate the influence of N_2O and CH_4 on the overall GHG balance of the growing bioenergy crops, we have extrapolated from the arithmetic mean of the 12 monthly measurements of each year and averaged over the total measurement period. To do so, soil fluxes of N_2O and CH_4 have been converted into CO_2 equivalents, taking into account their global warming potential over the 100 year time horizon (IPCC, 2007). Expressed in CO_2 equivalents, N_2O emissions were 152 kg CO_{2eq} ha^{-1} yr^{-1} from *Miscanthus*, 8 kg CO_{2eq} ha^{-1} yr^{-1} from willow and 339 and 1326 kg CO_{2eq} ha^{-1} yr^{-1} from ACA and ACB respectively (Fig. 2a). CH_4 emissions expressed as CO_2 equivalents were −5 and −14 kg CO_{2eq} ha^{-1} yr^{-1} from *Miscanthus* and willow and −11 and −9 kg CO_{2eq} ha^{-1} yr^{-1} from ACA and ACB respectively (Fig. 2b). Annual soil respiration rates were calculated as 20 000 kg CO_2 ha^{-1} yr^{-1} for *Miscanthus*, 26 000 kg CO_2 ha^{-1} $yr^{-1}$for willow, 19 000 and 25 000 kg CO_2 ha^{-1} $yr^{-1}$for the annual crops ACA and ACB respectively (Fig. 2c).

No clear seasonal trends in N_2O or CH_4 emissions were observed. However, peak N_2O emissions from the annual crops may be attributed to fertilizer application in spring, and harvest in autumn. There was no significant correlation between N_2O fluxes and soil moisture or soil respiration for the bioenergy or the annual crops. Methane fluxes were very small in general, and no clear correlations with soil respiration could be established. Methane was generally only emitted at larger soil moisture contents (>30% v/v, WFPS > 52–72%). On the *Miscanthus* field, a significant relationship between CH_4 flux and moisture content was observed ($P < 0.001$, $R^2 = 0.371$, Fig. 3).

There was no clear correlation between soil temperature and N_2O flux, however, N_2O uptake and emission rates were larger when the soil temperature was >15°C (Fig. 4). Soil respiration rates from the bioenergy crops also peaked at around 15°C when the most scatter occurred. This was more pronounced for *Miscanthus* than willow. For the bioenergy crops, a trend of larger soil respiration rates with higher soil temperature (at 10 cm depth) was observed. In the *Miscanthus* field, the peak soil temperature of 16°C on the 19.06.2009 corresponded to the largest mean respiration rate of 0.56 g m^{-2} h^{-1}. In the willow field, soil temperatures were usually slightly higher during summer than in *Miscanthus*. The maximum soil temperature of 20°C recorded here on 22.09.2009 corresponded to the largest mean soil respiration rate of 0.38 g m^{-2} h^{-1}.

Air temperature did not correlate well with N_2O fluxes for any of the crops, but there was a tendency towards lower N_2O emissions at higher temperatures

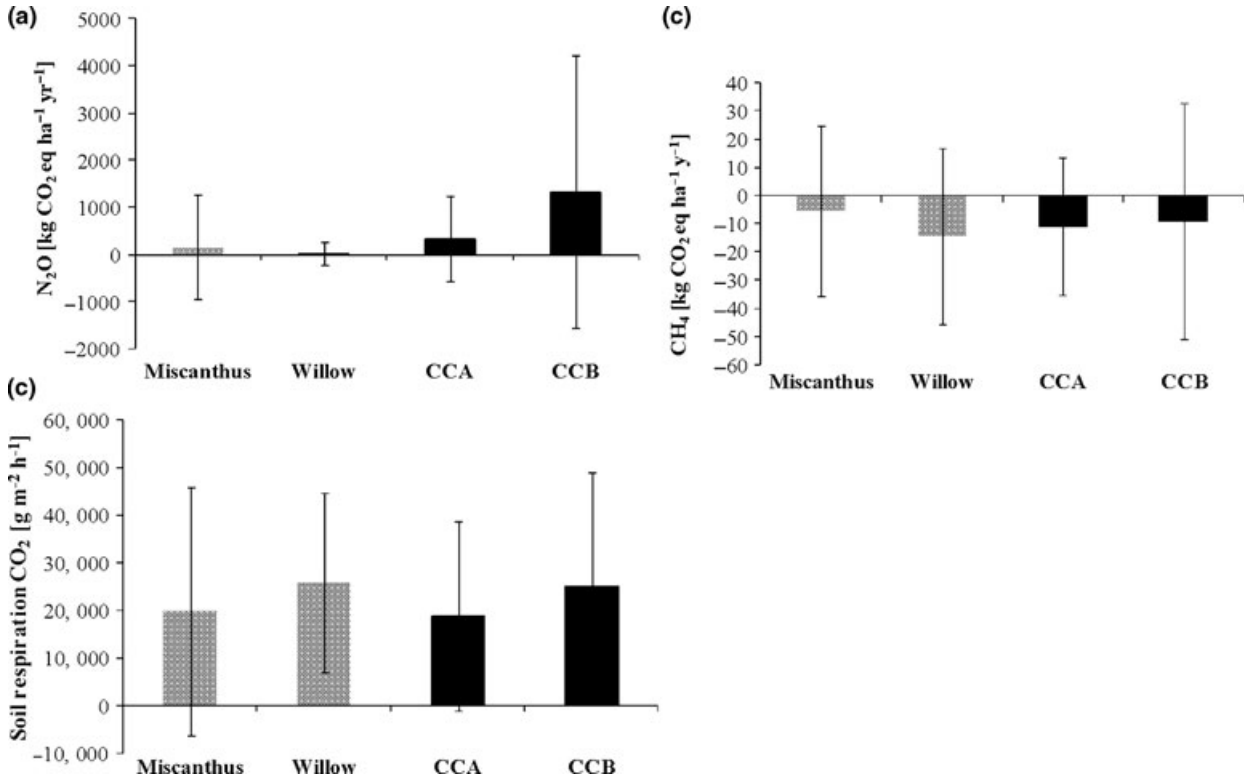

Fig. 2 Annual (a) CH_4, (b) N_2O fluxes and soil respiration rates (c) expressed in CO_2 equivalents. Error bars are standard deviations from replicate chambers per plot (n = 5).

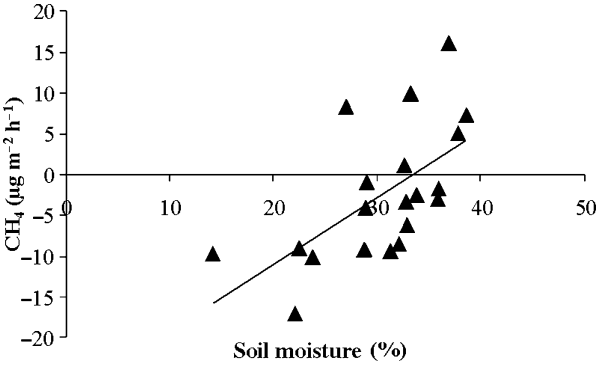

Fig. 3 Correlation between CH_4 emissions from *Miscanthus* and soil moisture (%). ($P < 0.001$, $R^2 = 0.371$).

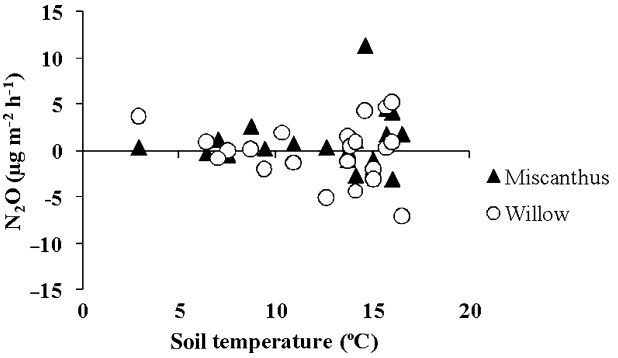

Fig. 4 Correlation between mean N_2O emissions from *Miscanthus* and willow and soil temperature at 10 cm depth.

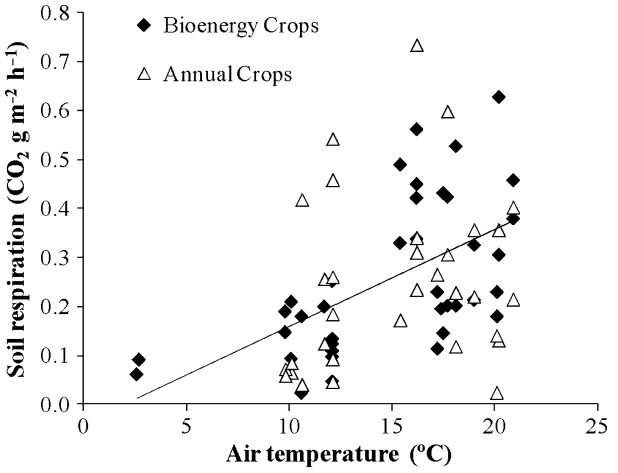

Fig. 5 Correlation between mean CO_2 soil respiration rates from bioenergy and annual crops and mean air temperature (°C). The correlation was not significant for annual crops. ($P < 0.001$, $R^2 = 0.3389$ for bioenergy crops).

under willow. Furthermore, air temperature did not have a large effect on CH_4 emissions, apart from higher CH_4 uptake rate under *Miscanthus* at higher temperatures. With respect to air temperature, there was a trend towards higher soil respiration rate at higher temperatures for all crops with strongest relationships seen for the bioenergy crops (Fig. 5). The relationship between air temperature and soil respiration for the bioenergy crops was significant ($P < 0.001$, $R^2 = 0.3389$, Fig. 5). Again, the scatter was also highest between 15 and 20°C for all crops.

In 2009, the cumulative NEE of CO_2 flux in the *Miscanthus* measured by eddy covariance was −1280 kg ha^{-1} yr^{-1}. There were small gaps in the dataset which were not gap-filled, so this value is possibly a slight underestimate as the data gaps mainly occurred during summer when largest CO_2 uptake rates would be expected. Hence, the CO_2 flux is the largest of all the GHG fluxes in the perennial crops. However, when calculating a GHG budget for the growing *Miscanthus* and willow crops, for 2009 the carbon equivalent contribution of N_2O and CH_4 to the total GHG budget of the *Miscanthus* crop is evident (Table 3). The extrapolation of monthly CH_4 and N_2O fluxes to annual fluxes for 2009 was merely done to intercompare the soil GHG fluxes with the NEE.

To compare the NEE from the *Miscanthus* field from the 2 years of 2009 and 2010, the growing season period from May to October was chosen as NEE, data were available for both years during this time period. The NEE during this period (cumulative) was higher in 2010 totalling −210 g m^{-2} than 2009 totalling −136 g m^{-2} (Table 3).

Simultaneous NEE measurements from *Miscanthus* and SRC willow were only made for the period 7 May to 16 October 2010. Cumulative NEE fluxes for this short period were −770 g C m^{-2} h^{-1} for *Miscanthus* and −602 g C m^{-2} h^{-1} for SRC willow, suggesting that the uptake of carbon by the *Miscanthus* was greater than that of the SRC willow.

GHG budgets were calculated for this growing season (May to October 2010) (Table 3). In both crops, CO_2 uptake is the major flux with −210 g m^{-2} from *Miscanthus* and −164 g m^{-2} from willow. The next largest flux is N_2O which is a positive flux from *Miscanthus* (emission) with 1.9 g CO_2 eq m^{-2} and a negative flux (uptake) from willow totalling −7.01 g CO_2 eq m^{-2}. CH_4 has the smallest proportion (uptake) of the GHG budget in both bioenergy crops with −0.58 g CO_2 eq m^{-2} in *Miscanthus* and −1.23 g CO_2 eq m^{-2} in willow (Table 3).

In addition to the field observations of N_2O emissions from the 4 fields, we compared N fertilizer-induced N_2O and N_2 emissions from *Miscanthus* and ACB. Fertilization with NH_4NO_3 at an equivalent rate of 50 kg N ha^{-1} stimulated N_2O emissions from both fields (Fig. 6) in a similar manner.

Table 3 Greenhouse gas (GHG) budgets for *Miscanthus* (M) and willow (W) in 2009 and comparison of growing seasons 2009 and 2010

	Crop	Year	N_2O	CH_4	CO_2	Sum
Annual budget for 2009						
GHG (g m^{-2} yr^{-1})	M	2009[a]	0.09	−0.03	−128	−102.23
GWP (g CO_2 eq m^{-2} yr^{-1})			26.41	−0.64	−128	
GHG (g m^{-2} yr^{-1})	W	2009[b]	−0.003	−0.03	ND	
GWP (g CO_2 eq m^{-2} yr^{-1})			−0.86	−0.78	ND	
Growing season 2009						
GHG (g m^{-2} 6 $month^{-1}$)	M	2009[b]	0.09	−0.02	−136	−109.58
GWP (g CO_2 eq m^{-2} 6 $months^{-1}$)			26.95	−0.53	−136	
GHG (g m^{-2} 6 $months^{-1}$)	W	2009[b]	−0.004	−0.02	ND	
GWP (g CO_2 eq m^{-2} 6 $months^{-1}$)			−1.19	−0.51	ND	
Growing season 2010						
GHG (g m^{-2} 6 $months^{-1}$)	M	2010[c]	0.006	−0.02	−210	−208.68
GWP (g CO_2 eq m^{-2} 6 $months^{-1}$)			1.9	−0.58	−210	
GHG (g m^{-2} 6 $months^{-1}$)	W	2010[c]	−0.02	−0.05	−164	−172.24
GWP (g CO_2 eq m^{-2} 6 $months^{-1}$)			−7.01	−1.23	−164	

[a]Whole year with 6% missing, nongapfilled, for CO_2, so likely to be a slight underestimation.
[b]7 May to 16 October subset of 2009 annual data (†) to compare with the equivalent period in 2010.
[c]7 May to 16 October.
ND, not determined.

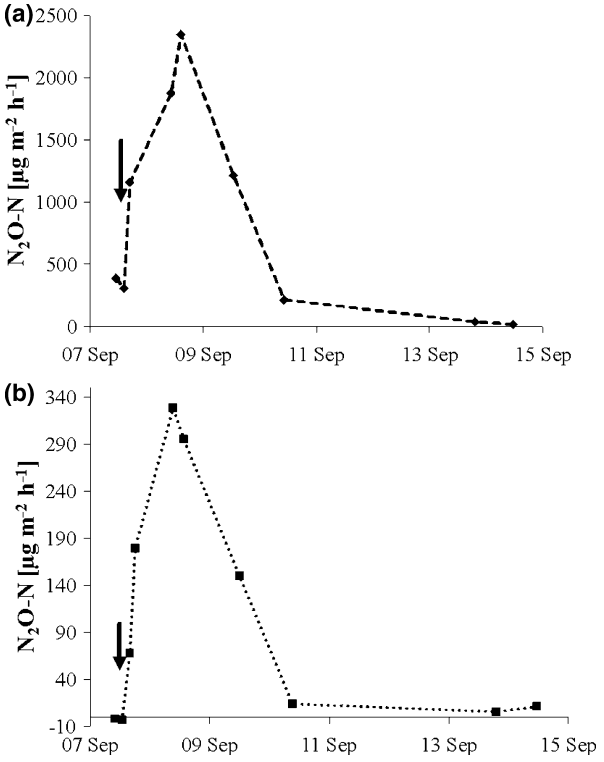

Fig. 6 Nitrous oxide (N_2O) stimulation by fertilization with NH_4NO_3 (50 kg N ha^{-1}) of soil under oilseed rape (ACB) (a) and the bioenergy crop *Miscanthus* (b). The arrows indicate the fertilizer application. (Note different scales on y-axes in Fig. 7a and b).

Before fertilizer application, N_2O emissions were around zero from *Miscanthus* and around 300 µg m^{-2} h^{-1} from oilseed rape, which ties in with the monthly measurements taken from these crops from the normally sampled 20 chambers which were also sampled during the time of the fertilizer application experiment. Within less than 24 hours, N_2O emissions started to rise from both fields. Maximum N_2O emissions were measured 36 hours after fertilizer application and for the oilseed rape plots it reached 2350 µg m^{-2} h^{-1}, whereas for *Miscanthus* plots it only reached 330 µg m^{-2} h^{-1}, which is about 7 times smaller than that of soil under oilseed rape. From then on emissions declined. At the end of the experiment after the fertilizer peak had been emitted, no significant difference could be seen between the crops, emissions from *Miscanthus* were slightly positive (around 10 µg m^{-2} h^{-1}) and emissions from oilseed rape were around 17 µg m^{-2} h^{-1}. The first response to the fertilizer application was noticed in the form of N_2O emission, N_2 emissions occurred later and peak emissions were measured a week after fertilizer application for both *Miscanthus* and oilseed rape, in very similar orders of magnitude (Fig. 7). The cumulative fluxes over 8 days (n = 10) from *Miscanthus* were 0.01 g N m^{-2} of N_2O and 4.73 g N m^{-2} of N_2 and 0.15 g N m^{-2} of N_2O and 4.48 g N m^{-2} of N_2 from oilseed rape (ACB). Hence, the N_2O emissions were an order of magnitude higher from oilseed rape, but N_2 emissions were similar for both crops.

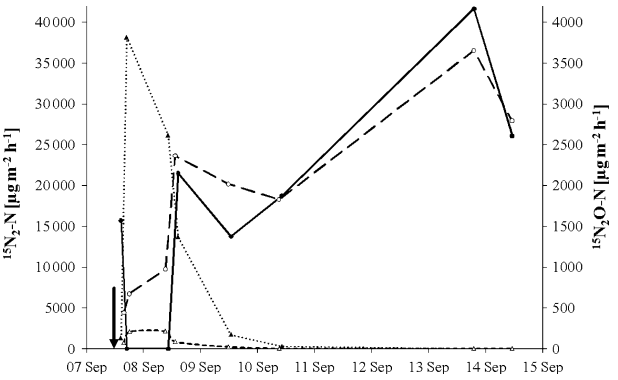

Fig. 7 Nitrous oxide (N_2O) (right hand *y*-axis) and N_2 (left hand *y*-axis) flux stimulation by fertilization with $NH_4{}^{15}NO_3$ (equivalent of 50 kg N ha^{-1}) of soil supporting the annual crop oilseed rape (filled circles, black line = N_2; filled triangle, dotted line = N_2O) and soil supporting the bioenergy crop *Miscanthus* (unfilled circles, broad dashed line = N_2; unfilled triangles, dashed line = N_2O). The arrow indicates the date of ^{15}N fertilizer application. Note the different axis scales.

During the experiment (07–15 September 2010), the volumetric soil moisture content was 30–35% (WFPS 65–76%) in the *Miscanthus* with 35–40% (WFPS 85–97%) slightly higher in the oilseed rape. Soil moisture increased slightly in both crops after fertilization, because it was applied in solution. From then onwards, the soil dried out slightly with soil moisture generally being slightly lower under the *Miscanthus* [mean 30%, min 26%, max 38%, WFPS 65(56–82)%] than the oilseed rape [mean 33%, min 24%, max 45%, WFPS 80(58–109)%]. During the fertilization experiment, mean soil respiration rates in the *Miscanthus* ranged from 0.09 to 0.17 g m^{-2} h^{-1} and did not change much, even after the fertilization event. On the other hand, soil respiration from the oilseed rape soil showed an initial burst after fertilization with mean peak emissions of 2.33 g m^{-2} h^{-1} which then declined to about 0.5 g m^{-2} h^{-1} for a few days, then rose to between 1 and 1.5 g m^{-2} h^{-1} at the end of the experiment. Soil respiration was generally higher from oilseed rape than *Miscanthus* throughout the experiment (Fig. 8).

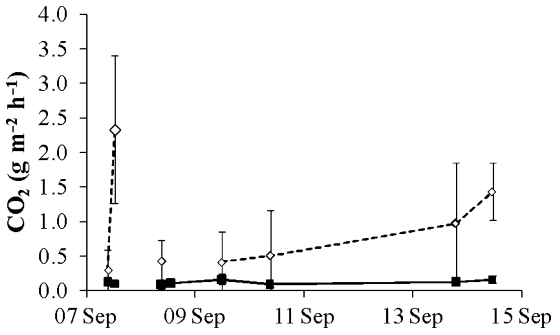

Fig. 8 Soil respiration during the fertilization experiment from oilseed rape (open diamonds, dashed line) and *Miscanthus* (filled squares, solid line). Error bars are standard deviation.

Fertilization with $NH_4{}^{15}NO_3{}^-$ increased concentrations of ^{15}N-NO_3 up to threefold higher in soil under oilseed rape than in soil under *Miscanthus* (Fig. 9). The maximum measured concentration of ^{15}N-NO_3 from oilseed rape was 8.4 µg g^{-1} soil and 2.5 µg g^{-1} soil from *Miscanthus*. These peak concentrations occurred a day after the fertilization in the oilseed rape field, but on the same day, a few hours after fertilization in the *Miscanthus* field. After the initial peak, concentrations gradually declined in both crops. Ammonium (^{15}N-NH_4) concentrations were generally lower with maximum concentrations of 0.55 µg g^{-1} soil from oilseed rape, 4 days after fertilization and 1.7 µg g^{-1} soil from *Miscanthus*, 1 day after fertilization. In the *Miscanthus* field, after the initial peak, concentrations of ^{15}N-NH_4 gradually declined whereas under oilseed rape ^{15}N-NH_4 rose after fertilization and stayed at about the same low level.

Discussion

Compared with the few published field measurement data, N_2O fluxes measured from *Miscanthus* in this study were in the same order of magnitude as cumulated growing season fluxes of 0.008 g m^{-2} (April/May) and 0.014 g m^{-2} (June to August) reported from an unfertilized *Miscanthus* field in Denmark (Jørgensen *et al.*, 1997). Likewise, published N_2O fluxes from *Miscanthus* in Japan (Toma *et al.*, 2010) of 0.005 g m^{-2} in the growing season of 2008 and 0.023 g m^{-2} in 2009 agree well with our growing season fluxes of 0.09 g m^{-2} in 2009 and 0.006 g m^{-2} in 2010 from *Miscanthus*, and show that the variation between years can be quite large. The same authors report zero to slightly negative CH_4 fluxes which also agrees well with our measurements. The positive measured CH_4 fluxes here are still very small fluxes, but due to the clayey nature of the soil, WFPS can be high at times and might contribute to these small positive fluxes.

A large N_2O sink of 350–428 g CO_2 eq m^{-2} yr^{-1} as simulated with the DAYCENT model and reported from the US (Davis *et al.*, 2010b) is not in agreement with our results; our measured fluxes were generally small, but on average positive (source). Their model simulations were parameterized with data from *Miscanthus* trial plots in Europe and Illinois, however, details of that data were not mentioned. Differences between their study and results presented here are most likely due to different soil and climatic properties. In this modelling study, (Davis *et al.*, 2010b) it was also

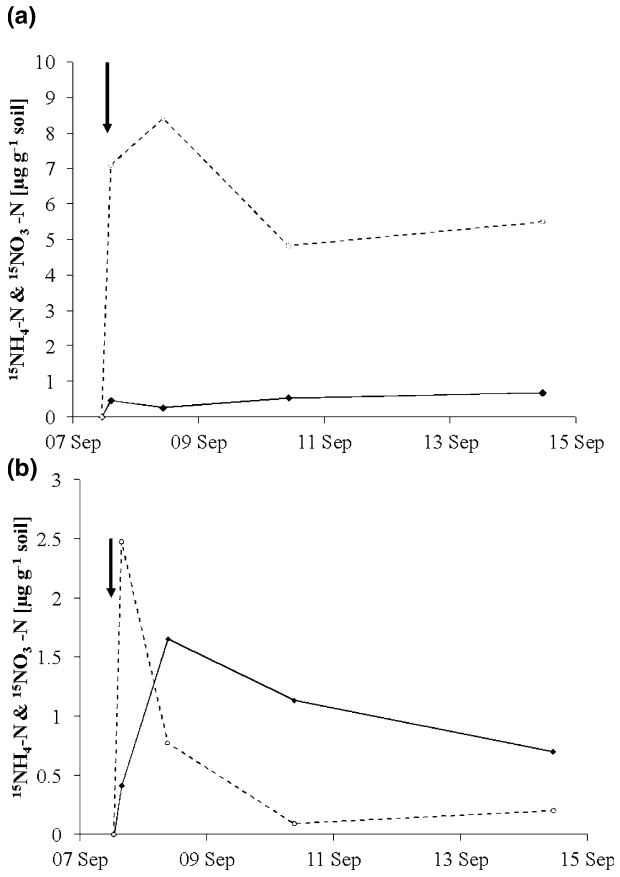

Fig. 9 Changes in ^{15}N-NH_4^+ and -NO_3^- concentrations after fertilization with $NH_4{}^{15}NO_3$ (50 kg N ha^{-1}) in the annual crop oilseed rape (a) and the bioenergy crop *Miscanthus* (b). The arrow indicates the fertilizer application.

reported that if the hypothesis that *Miscanthus* relies on N-fixation to meet its N requirement is correct, *Miscanthus* would be a net GHG sink averaged over 5 years. It would be neither sink nor source when averaged over a 10 year period.

The N_2O emissions measured in Lincolnshire were a lot higher from annual crops, possibly due to the use of N fertilizer in spring and tillage in early autumn, than bioenergy crops, which were not fertilized or tilled during this study period. However, fertilizer-induced higher N_2O emissions from *Miscanthus* were measured during a field experiment which is in agreement with reported emissions from a fertilized *Miscanthus* plot in Denmark (Jørgensen *et al*., 1997) which reached a maximum of 155 µg m^{-2} h^{-1} (although it is not clear how soon after fertilization, this was measured). Peak emissions from *Miscanthus* in this study reached about 300 µg m^{-2} h^{-1} 36 h after fertilizer application.

Cumulative fluxes over 8 days during a fertilization experiment resulted in similar rates of N_2 fluxes from *Miscanthus* and oilseed rape, but N_2O fluxes were about an order of magnitude higher from oilseed rape than from *Miscanthus* (Fig. 7). Overall, the total denitrification rate (N_2O + N_2) was about the same in both crops. At the same time, the 20 usually sampled chambers in all four crops did not show any significant increase in emissions over time. It has been reported previously that a higher pH would increase N_2 production (Burth & Ottow, 1983). The pH in the Miscanthus field was 7.13 ± 0.02whereas the pH in the oilseed rape field was 6.45 ± 0.09 which supports this theory.

In addition, the soil under the annual crops is regularly fertilized with N as part of routine crop management. However, the *Miscanthus* soil had not received any N fertilizer in the last 6 years. Such differences might result in a different microbial composition leading to different N_2O emission rates, however, this remains to be tested. As the N_2 emissions from denitrification were at a similar rate for *Miscanthus* and oilseed rape, but N_2O emissions and soil NO_3 concentrations were lower from the *Miscanthus* than oilseed rape soil, the main fate of the applied ^{15}N in the *Miscanthus* would be plant and microbial uptake, i.e. immobilization. Even though the NO_3 was labelled with ^{15}N in the applied fertilizer, some ^{15}N-NH_4 was recovered in both soils at similar concentrations which may suggest that ammonification from NO_3^- to NH_4^+ also occurred (Acton & Baggs, 2011).

Soil N_2O, CH_4 and respiration fluxes in relation to soil or air temperatures often showed highest emissions and the greatest scatter around 15 °C, which is also the upper end of the recorded temperatures. It is likely that the microbial activity is highest around this temperature which will influence emission processes as well as uptake processes in a similar way, resulting in a high scatter of observed fluxes, i.e. maximum and minimum GHG fluxes occurring at the same (higher) temperatures (King & Adamsen, 1992; Davidson *et al*., 1998). Uptake of N_2O by soils is occasionally observed and has been reported from many different studies (Chapuis-Lardy *et al*., 2007). Figure 4 shows clearly how microbial activity responsible for N_2O production and consumption processes increases as temperature increases. As none of the correlations were very strong for all crops, the main difference between the bioenergy crops and the annual crops, which might explain the difference especially in N_2O emissions, was the N-input as fertilizer. Moreover, plant micro-climate did not result in differences in soil temperature and microbial activity amongst the various crops.

According to the results of this field study, the GHG savings by growing bioenergy crops possibly depend on the rate of N fertilization. As the main difference between annual and perennial bioenergy crops investigated in this study was the fertilizer application, the

conclusion is based on two different aspects, the long-term measurements and the short-term fertilizer-application experiment. Land resources in the UK and Europe will limit the contribution that bioenergy crops can make to achieve the renewable energy targets (Rowe *et al.*, 2009) and fertilizer might be necessary to achieve the yields needed to reach those targets. Although, several experimental studies have also shown that Miscanthus yields do not improve with N fertilization (Himken *et al.*, 1997; Clifton-Brown *et al.*, 2007; Danalatos *et al.*, 2007).

Overall *Miscanthus* still appears to be a low N-demanding crop compared with other crops. Reasons for this, in addition to the above mentioned biological N-fixation are severalfold. (1) *Miscanthus* nutrients are returned to the soil as litter as only the stems of the crop are harvested and any leaf litter remains on the soil. There is also some evidence of high N re-translocation of aboveground tissues to belowground, prior to senescence (Beale & Long, 1997; Beuch *et al.*, 2000). (2) In the *Miscanthus* establishment phase, the plants benefit from residual N in the soil from the previous crop. (3) *Miscanthus* does in fact receive some additional N through atmospheric deposition, which in this area accounts for about 12 kg ha^{-1} yr^{-1} (R. Smith, personal communication). However, nutrients are removed with every harvest and it is questionable whether high yields can be sustained without fertilizer input in the long-term. The results from this study clearly show that N_2O emissions, will increase when adding N fertilizer and would offset the GHG balance and make the crop less GHG neutral.

The results presented here show that N_2O emissions from the two bioenergy crops are significantly smaller ($P < 0.0001$) than that from the adjacent annual crops. On the other hand, CH_4 emissions and soil respiration from bioenergy crops are not significantly different from emissions from annual crops. At this stage, we cannot make a statement about NEE, as it was not measured over the annual arable crops and cannot be estimated easily. However, some studies have shown that even arable soils sequester CO_2 (Davis *et al.*, 2010a), and perennial vegetation might sequester more than annual crops (Shurpali *et al.*, 2009). Therefore, we can accept our hypothesis that the GHG balances are different and very likely to be smaller from the perennial bioenergy crops *Miscanthus* and willow than annual arable crops. As this difference appears to be due to N fertilizer application, this statement is only valid for nonfertilized bioenergy crops. As soon as N fertilizer is applied to bioenergy crops like *Miscanthus*, the difference between bioenergy and annual crops will be smaller. Moreover, potential N_2O emissions will most likely also be proportional to the amount of fertilizer applied, as already known from various agricultural studies.

Currently the energy crops in the UK are not generally fertilized with N, but it already appears to be standard practice e.g. in Ireland. On the other hand, due to 20 year life time of the bioenergy crops and seasonal and interannual variations (soil & climate) it is important to continue measurements long-term before the on field GHG footprint of the bioenergy crop can be properly evaluated. It has been shown in this study that the GHG balance changes from year to year and the driving factors need to be determined in more detail.

Conclusions

The outcome of this study is that perennial bioenergy crops are not 'GHG neutral', but appear to emit less N_2O than annual crops because they did not receive N fertilizer. However, our fertilization experiment has shown that if perennial bioenergy crops are to be fertilized in future, N_2O emissions will probably increase substantially which could offset the GHG balance more, and the GHG savings in comparison with annual crops would be even smaller.

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